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Naltrexone for treatment of impaired awareness of hypoglycemia in type 1 diabetes: A randomized clinical trial

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Abstract

Aims—Impaired awareness of hypoglycemia (IAH) is a limiting factor in the treatment of type 1 diabetes (T1D) and is a challenging condition to reverse. The objective of this study was to test the hypothesis that naltrexone therapy in subjects with T1D and IAH will improve counterregulatory hormone response and recognition of hypoglycemia symptoms during hypoglycemia.

Methods—We performed a pilot randomized double blind trial of 4 weeks of naltrexone therapy (n=10) or placebo (n=12) given orally in subjects with T1D and IAH. Outcome measures included hypoglycemia symptom scores, counterregulatory hormone levels and thalamic activation as measured by cerebral blood flow using MRI during experimental hypoglycemia in all subjects before and after 4 weeks of intervention.

Results—After 4 weeks of therapy with naltrexone or placebo, no significant differences in response to hypoglycemia were seen in any outcomes of interest within each group.

Conclusions—In this small study, short-term treatment with naltrexone did not improve recognition of hypoglycemia symptoms or counterregulatory hormone response during experimental hypoglycemia in subjects with T1D and IAH. Whether this lack of effect is related to

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the small sample size or due to the dose, the advanced stage of study population or the drug itself should be the subject of future investigation.

1. Introduction

Iatrogenic hypoglycemia is a common and feared complication of insulin therapy. Recurrent exposure to iatrogenic hypoglycemia in patients with insulin treated diabetes can lead to development of impaired awareness of hypoglycemia (1), a condition that is estimated to occur in 20% of patients with type 1 diabetes (2). Fear of hypoglycemia can limit the ability of patients with diabetes to achieve the glycemic control shown to prevent complications of diabetes. Strict avoidance of hypoglycemia has been shown to partially restore awareness of hypoglycemia; however it is very difficult to achieve and maintain over the long term (3–5). Consequently, there has been great interest in developing therapies that will prevent and/or reverse impaired awareness of hypoglycemia in diabetes.

One potential therapy to prevent or reverse impaired awareness hypoglycemia in diabetes may be opioid receptor antagonists. Endogenous opiates have been shown to modulate hormonal responses during hypoglycemia and may play a role in the development of impaired awareness of hypoglycemia (6). Intravenous administration of naloxone, an opioid receptor antagonist, during hypoglycemia has been shown to augment the counterregulatory response to hypoglycemia in dogs (7) and humans (8). When infused during antecedent hypoglycemia, naloxone has been shown to prevent development of defective counterregulatory hormone response to subsequent hypoglycemia in healthy humans (9) and in patients with type 1 diabetes (10). Whether chronic administration of an opioid receptor antagonist to patients with type 1 diabetes at risk for or suffering from impaired awareness of hypoglycemia will be an effective therapy remains unknown.

How to best test potential therapies for impaired awareness of hypoglycemia in patients with diabetes has not yet been determined. Patients with the condition are likely to gain most from the development of a successful therapy, but it isn’t clear that a drug that prevents hypoglycemia induced impairment in counterregulation will be successful in patients who already are impaired. Administration of a candidate drug to patients with type 1 diabetes who retain awareness of hypoglycemia might help us understand if the drug can prevent the development of impaired awareness, but the correct dosing regimen for chronic use must be defined. Whether the same drug can both prevent and treat impaired awareness of hypoglycemia in diabetes also remains unknown. In this report, we aim to test one approach with the intention of gaining insights that will guide the design of future trials testing drugs to prevent and treat this most challenging complication of insulin therapy in diabetes.

In this study, we examined the safety and efficacy of four weeks of oral administration of the opioid receptor antagonist naltrexone in participants with type 1 diabetes and hypoglycemia unawareness defined by the Cox questionnaire (11). The primary objective of this randomized placebo controlled trial was to test the hypotheses that naltrexone therapy in subjects with type 1 diabetes and impaired awareness of hypoglycemia will improve counterregulatory hormone responses, increase recognition of hypoglycemia symptoms, and increase brain activation during hypoglycemia in the thalamus, a region that is involved in

the brain response to hypoglycemia (12). The secondary objective was to gain insights into whether it would be better to test patients with type 1 diabetes who do or do not have awareness of hypoglycemia in future studies of candidate drugs for the prevention and treatment of this condition. If naltrexone fails to reverse the condition in a population of diabetic subjects with impaired awareness of hypoglycemia, future efforts involving use of opioid receptor antagonist should be focused towards prevention of the development of the condition in patients with type 1 diabetes who retain awareness of hypoglycemia.

2. Materials and Methods

2.1. Subjects

Otherwise healthy subjects with type 1 diabetes with HbA1C < 8% (64 mmol/mol), between the ages 18 and 65 years of age were recruited for participation. Type 1 diabetes was defined on clinical grounds. The Cox questionnaire (11) was used to categorize subjects with type 1 diabetes as having impaired awareness of hypoglycemia. Exclusion criteria included concomitant use of acetaminophen, aspirin or ibuprofen (all may increase risk of naltrexone induced liver dysfunction), history of liver disease, renal insufficiency, central nervous system or cardiac disease and presence of any characteristics that would preclude placement in the MRI magnet.

2.2. Study design and experimental protocol

The study was designed to assess the occurrence of hypoglycemia over one week in the free living state and responses to experimental hypoglycemia before and after a four week treatment period in which subjects were randomized to receive naltrexone for 4 weeks or an identically appearing placebo. The protocol was approved by the Institutional Review Board at the University of Minnesota.

Study participation began with a screening visit (visit 1), where subjects were educated in the use of the continuous glucose monitor and sent home with sufficient supplies to collect data for the seven days before the pre-treatment clamp study at visit 2. During this seven day period, subjects were also asked to check their blood glucose at a minimum before each of three meals and at bedtime and record any glucose reading <70 mg/dl on provided hypoglycemia log sheets. Subjects documented whether the hypoglycemia was recognized and treated by themselves or someone else and any associated symptoms they may have had. Hypoglycemia was defined as any episode of hypoglycemia that required the assistance of another to recognize/treat or any documented blood glucose < 70 mg/dl.

On the morning of the first hypoglycemia clamp study (visit 2), subjects presented to the Center for Magnetic Resonance Research after an overnight fast. After arrival, their continuous glucose monitor was removed and they were prepared for the insulin clamp study as previously described (12). Cerebral blood flow data was collected initially during euglycemia (blood glucose 95 mg/dl) and then again during hypoglycemia (blood glucose 50 mg/dl). Samples for counterregulatory hormones were collected at baseline and every 10–15 minutes during hypoglycemia. Symptoms of hypoglycemia were quantified using a previously validated questionnaire (13). Subjects were asked to score from 0 (none) to 6

(severe) on six autonomic symptoms (heart pounding, shaky/tremulous, nervous/anxious, sweaty, hungry, tingling) and six neuroglycopenic symptoms (difficulty thinking, tired/drowsy, weak, warm, faint, dizzy). At the completion of the clamp study, subjects were then sent home with their treatment assignment according to the randomization protocol.

An Investigational New Drug approval for the use of naltrexone was obtained from the Food and Drug Administration (#103409). The investigational pharmacy at the University of Minnesota ensured that the naltrexone and placebo tablets were identical in appearance and managed the randomization assignments. Assignment to naltrexone or placebo was blinded to participants and investigators until the study was completed. The dose of Naltrexone was titrated up over 10 days (25 mg daily × 5 days, then 50 mg daily × 5 days, then 50 mg twice daily × 18 days).

At 14 ± 1 days after the first episode of experimental hypoglycemia, subjects returned to provide blood for measurement of ALT, AST, CPK, and creatinine (visit 3). At visit 4 (7 days prior to the second hypoglycemia clamp study), the continuous glucose monitor was again placed and subjects were instructed to keep the hypoglycemia log for the next 7 days. On day 28 (Visit 5) a second hypoglycemia clamp and MRI study was performed. Subjects took their final dose of naltrexone/placebo on the morning of visit 5 and presented to repeat the clamp study with measurement of cerebral blood flow as described above for visit 2. The full study protocol is depicted in Figure 1.

2.3. Laboratory analyses

Blood samples for counterregulatory hormones obtained during the hypoglycemia protocol were send to Vanderbilt Diabetes Research and Training Center (DRTC), Hormone Assay and Analytical Services Core for analysis. Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography (Dionex, formerly ESA, Inc.). Plasma growth hormone and cortisol were measured by radioimmunoassay (Diagnostic Products Corporation, Inc). Plasma glucagon was measured by radioimmunoassay (Modified Millipore, Merck).

2.4. Continuous glucose monitor analysis

A Dexcom Seven Plus continuous glucose monitor was provided to subjects to wear for 7 days prior to each clamp study. Subjects were blinded to the monitor's glucose readings. Cumulative exposure to hypoglycemia was determined using AUC for the glucose curve over time spent with interstitial glucose < 70 mg/dl. Subjects who were previously using a personal continuous glucose monitor and declined to use the study device were allowed to continue using their own monitor (not blinded to their glucose readings), but their AUC data were not available for analysis.

2.5. MRI acquisition and processing

MRI measurements were performed using a 3.0 Tesla Siemens Trio scanner (Siemens, Erlangen, Germany), using imaging and processing tools utilized previously (12). Measurements of cerebral blood flow in the midbrain regions were obtained during euglycemia and during hypoglycemia using the technique of arterial spin labeling as

previously described (14). Thalamic brain activation was calculated by the difference in the signal intensity (SI) of the cerebral blood flow images for hypoglycemia minus euglycemia, relative to euglycemia (here named Δ_{SI} (%)), averaged over the thalamus of each subject as outlined in the common normalized Tailarach space.

2.6. Statistical analysis

Data were summarized using mean±standard deviation (SD) and percent as appropriate. Hypoglycemia symptom scores, counterregulatory hormone levels (average observed over three blood samples collected during hypoglycemia for each subject), AUC of glucose less than 70 mg/dl, and cerebral blood flow outcomes were analyzed using paired t-test for the comparison of pre- to post-treatment within each group. Poisson regression was used to analyze the number of hypoglycemia episodes; proportion of hypoglycemia episodes with symptoms was analyzed using logistic regression. Since each participant provided pre and post-treatment counts and proportions, the robust sandwich variance based on Generalized Estimating Equations was used. Because of the small sample size, tests were repeated using non-parametric tests; since the results were consistent with the parametric tests, they are not included here. Clinical study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at the University of Minnesota (15).

3. Results

3.1. Baseline characteristics

30 subjects with type 1 diabetes and impaired awareness of hypoglycemia were enrolled. Six subjects could not complete the first hypoglycemic clamp study and were excluded. 24 subjects with type 1 diabetes and impaired awareness of hypoglycemia underwent randomization. 2 subjects in naltrexone group were excluded due to incidental abnormal findings on brain MRI. 22 subjects (10 in the naltrexone group and 12 in the placebo group) completed 4 weeks of therapy. Table 1 shows the baseline characteristics of these 22 subjects who completed 4 weeks of therapy. Age, gender, body mass index, HbA1C, Cox score and duration of diabetes were similar between the naltrexone and placebo groups (Table 1).

3.2. Counterregulatory hormones and hypoglycemia symptom scores during Insulin clamp

There was no significant difference in mean blood glucose during the euglycemic clamp in pre- compared to post-treatment studies in both naltrexone (90±6 vs. 93±7 mg/dl, P=0.2) and placebo groups (93±6 vs. 95±4 mg/dl, P=0.3). Mean blood glucose was also similar during the hypoglycemic clamp before and after treatment in both naltrexone (49±5 vs. 49±3 mg/dl, P=0.8) and placebo (49±3 vs. 48±3 mg/dl, P= 0.7) groups. 20 subjects (9 in the naltrexone group and 11 in the placebo group) completed both pre and post-treatment clamp studies. Compared to the values collected during the pre-treatment hypoglycemic clamp, after 4 weeks of therapy with naltrexone or placebo there were no significant changes in epinephrine, cortisol, growth hormone and glucagon levels during hypoglycemia (Figure 2). Norepinephrine was lower (P=0.04) during the post-treatment clamp in the naltrexone group.

After 4 weeks of naltrexone or placebo therapy there was no significant change in total, adrenergic, or neuroglycopenic symptom scores during hypoglycemia as compared to symptoms scores during pretreatment hypoglycemia for either group (Table 2).

3.3. Hypoglycemic events

Patient-reported hypoglycemic events during the week before starting naltrexone or placebo and during the last week of therapy are shown in table 2. Results of patient-reported hypoglycemic events were available from all 22 subjects who completed the 4 weeks of therapy. Compared to the pretreatment week, there was no significant change in frequency of hypoglycemic episodes during the last week of treatment for either naltrexone or placebo (Table 2). 16 subjects used study provided continuous glucose monitor, whereas 6 subjects (4 in the naltrexone group and 2 in the placebo group) continued to use their personal continuous glucose monitor. Complete continuous glucose monitor results for the periods of one week before and during the last week of therapy were available from 7 subjects in the placebo and 5 in the naltrexone arm who used a study-provided device. There was no change in AUC <70 mg/dl over 7 days of continuous glucose monitoring between data collected before or after either treatment.

3.4. Cerebral blood flow outcomes

The hypoglycemia-induced cerebral blood flow changes (i.e. Δ_{SI} (%)) in the thalamus were not significantly different between pre- and post-treatment for both the placebo (1.3±3.4 vs 1.7±1.9%, P=0.6) and the naltrexone (2.4±2.5 vs 1.4±3.6% P=0.5) group, indicating that the treatment did not induce a measurable effect on the thalamic activation above the sensitivity threshold of our MRI method (Figure 3).

3.5. Tolerability of naltrexone

Naltrexone was well tolerated. Three subjects on naltrexone reported headache or diarrhea but symptoms were mild and resolved without discontinuation of naltrexone. Mean ALT, AST and CPK levels remained unchanged after 4 weeks of therapy in both naltrexone and placebo groups.

4. Discussion

To our knowledge this is the first clinical trial of orally administered naltrexone to treat impaired awareness of hypoglycemia in patients with type 1 diabetes. Our results demonstrate that this dose of naltrexone given orally for 4 weeks was without significant effects on the frequency of hypoglycemia in real life or on symptom and counterregulatory hormone responses during experimental hypoglycemia. Naltrexone administration also did not show any effect on hypoglycemia-induced thalamic activation in this patient population.

These results are contrary to what we expected since previous studies have suggested endogenous opioids may be involved in the development of impaired awareness of hypoglycemia in type 1 diabetes (6, 7, 9, 10). The exact mechanism through which endogenous opiates might impair the counterregulatory response to hypoglycemia is unknown. Opiate receptors are expressed in the ventromedial hypothalamus and could

potentially modulate glucose sensing during hypoglycemia (16). In mice, preservation of counterregulatory responses during hypoglycemia with naloxone was associated with induction of genes in the hypothalamus that are thought to induce metabolic switching from carbohydrate to fat metabolism (17), suggesting that naloxone may preserve counterregulatory response during hypoglycemia by promoting use of alternate fuels instead of glucose.

Previous studies demonstrated the beneficial effects of naloxone in preventing the development of impaired awareness of hypoglycemia in humans when administered during antecedent episodes of experimental hypoglycemia (9, 10). In our study we administered naltrexone for a four-week period to patients with type 1 diabetes and impaired awareness of hypoglycemia, with the expectation that the drug would reduce or eliminate the impact of hypoglycemia experienced during the treatment period on the counterregulatory responses to subsequent episodes of hypoglycemia. The study subjects clearly experienced hypoglycemia during the treatment period, but the presence of naltrexone during this period had no impact on the counterregulatory responses to the experimental hypoglycemia performed at the end of the period. One potential reason our study did not find an effect is that the dose of oral naltrexone may have been insufficient to achieve the drug concentration achieved with intravenous naloxone. The naltrexone dose used was selected because of its known safety as a chronic treatment. It is possible that administration of naltrexone or another opioid antagonist using a different dosing schedule would be more effective in reversing impaired awareness of hypoglycemia in this patient population.

Another reason we may have failed to see an effect is that our study group of subjects with long standing type 1 diabetes and impaired awareness of hypoglycemia may have progressed to a state of impaired awareness that cannot be reversed by an opioid antagonist. As noted above, clinical efforts to reverse impaired awareness of hypoglycemia have had only limited benefit (3–5), perhaps because this condition cannot be reversed in all patients. This study provides important insights into designing future clinical trials to test drugs with aim of preventing or reversing impaired awareness of hypoglycemia in patients with diabetes. Previous studies in humans have evaluated the role of opioid antagonists (9, 10) in preventing development of impaired awareness of hypoglycemia during antecedent episodes of experimental hypoglycemia usually over 1–2 days. Patients with long standing diabetes and impaired awareness of hypoglycemia are at greater risk of severe hypoglycemia and would likely benefit the most from development of a therapy for this condition. In this study we tested the approach of using short-term oral naltrexone therapy in patients who had impaired awareness of hypoglycemia along with long standing diabetes. Future studies are needed to test if longer duration of therapy with oral naltrexone could successfully reverse impaired awareness of hypoglycemia. Future investigation of patients with type 1 diabetes of shorter duration who do not have impaired awareness of hypoglycemia may demonstrate that drugs in this class can prevent them from developing this condition, even if exposed to hypoglycemia as a complication of their insulin therapy. If so, it may be that drugs of this class could be used during or after exercise when the risk of hypoglycemia may be greatest to prevent the patient from developing impaired awareness of hypoglycemia to hypoglycemia experienced in subsequent days. Naltrexone was well tolerated in our study with few mild and self-limited adverse effects which did not require stopping the

medication. This may suggest that if future studies demonstrate naltrexone's effectiveness, patients could incorporate this treatment into their regimen without anticipating serious side effects. Based on the variability observed in our study sample (standard deviation of 36 pg/ml [placebo] and 65 pg/ml [naltrexone] in the pre- vs. post-treatment within-person difference in average epinephrine during hypoglycemia), a future clinical trial designed for 85% power and two-tailed type I error of 5% would need 25 per group to detect a group difference in epinephrine of 46 pg/ml, 100 to detect 23 pg/ml, or 200 to detect 16 pg/ml.

Previous studies using neuroimaging techniques have identified brain regions that are activated in response to hypoglycemia. In our previous work, we found that hypoglycemia induced thalamic activation measured by cerebral blood flow was blunted in subjects with type 1 diabetes and impaired awareness of hypoglycemia as compared with healthy controls (12). We hypothesized that thalamic activation in response to hypoglycemia would increase in subjects with type 1 diabetes and impaired awareness of hypoglycemia after 4 weeks of naltrexone therapy. However, in this study we did not see any significant increase in thalamic activation during hypoglycemia after 4 weeks of therapy with naltrexone or placebo.

The generalizability of results from this study is limited by small sample size. Another limitation of our study is that we did not have complete sets of continuous glucose monitor data as outcome measures from all of our participants. In clinical practice it is common for patients with type 1 diabetes and impaired awareness of hypoglycemia to use continuous glucose monitors. We were concerned about the safety of asking patients to stop using their own personal continuous glucose monitor so they could wear the blinded study monitor. We were only able to collect blinded continuous glucose monitor data on about half of our participants, which reduced the power we had to find an impact of naltrexone on this outcome.

In conclusion, in this small study, short-term treatment with naltrexone for 4 weeks did not reverse impaired awareness of hypoglycemia in patients with type 1 diabetes who had this condition. Future studies will be necessary to determine if a different dosing strategy or if naltrexone treatment in a group of patients less affected by impaired awareness of hypoglycemia is effective in preventing or treating this devastating complication of diabetes therapy.

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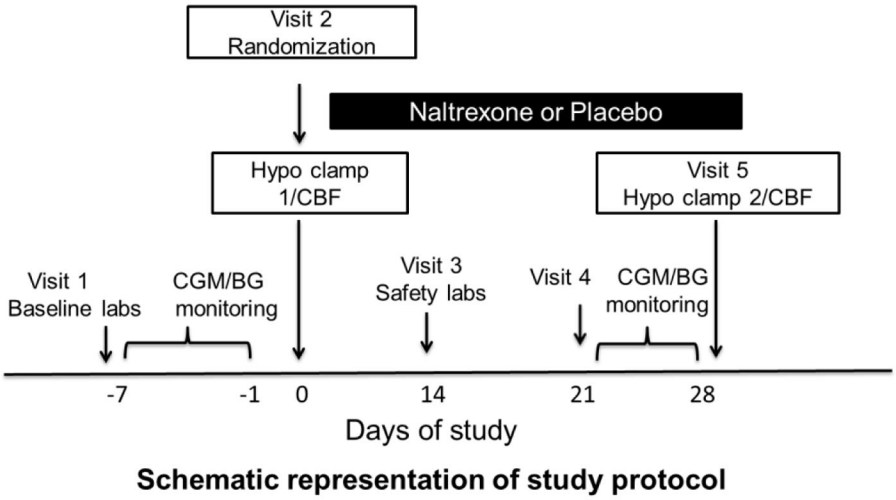

Figure 1.

Schematic representation of study protocol. CGM (continuous glucose monitoring), BG (blood glucose), Hypo (hypoglycemia), CBF (cerebral blood flow measurement)

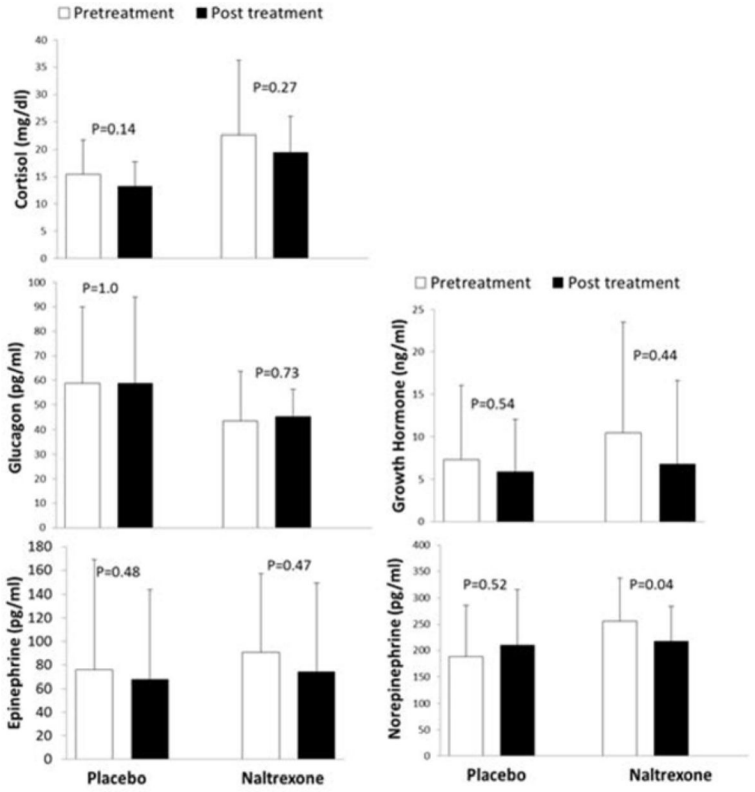

Figure 2.

Counterregulatory hormones during pre- and post-treatment hypoglycemic clamps. Data presented as mean ± SD.

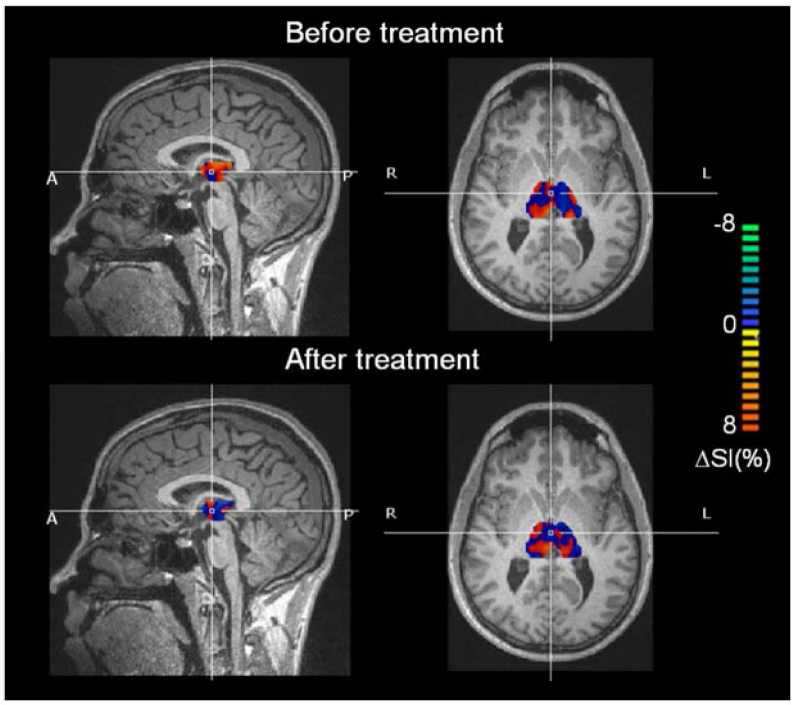

Figure 3.

Thalamic cerebral blood flow response to hypoglycemia before (top row) and after treatment (bottom row), shown on sagittal (left column) and axial slices (right column) from one representative subject. The cerebral blood flow response is identified by $\Delta_{SI}(\%)$. The cerebral blood flow response to hypoglycemia was averaged over the thalamic region per each subject, which were then used for statistical comparisons within and among groups.

Table 1

Subject characteristics

	Placebo	Naltrexone
Female/male	6/6	6/4
Age (years)	39±10	47±13
BMI (kg/m^2)	27±5	28±4
HbA1c (%)	6.5±0.7	7±0.5
HbA1c (mmol/mol)	48±7.7	53±5.5
Duration of diabetes (years)	25±11	32±13
Cox score	5.8±0.9	5.3±0.9

Data presented as number or as mean ± SD.

Table 2

Symptom scores during hypoglycemic clamps, patient reported hypoglycemic events and AUC <70 mg/dl per continuous glucose monitor over 7 days, pre- and post-treatment with naltrexone or placebo

	Naltrexone			Placebo		
	Pre-treatment	**Post-treatment**	**P-value**	**Pre-treatment**	**Post-treatment**	**P-value**
Total Hypo Symptom Score	17.7±15.1	14.5±12.2	0.5	15.3±12.1	12.6±7.5	0.5
Adrenergic symptom score	9.6±9.4	6.5±6.7	0.2	8.2±8.7	5.8±4.4	0.3
Neuroglycopenic symptom score	8.3±5.9	8±7.2	0.9	7.1±4.9	6.8±4.8	0.9
Total hypo episodes	6.3±4.4	4.3±5.2	0.10	8.0±5.3	6.6±4.1	0.20
Hypo with symptoms (Number of episodes)	2.0±1.6	1.4±1.1	0.31	2.6±2.9	2.4±3.0	0.85
Hypo without symptoms (Number of episodes)	4.3±4.9	2.9±4.6	0.17	5.4±3.7	4.2±3.1	0.13
Hypo with symptoms (% of episodes)	34.9±29.3	52.8±40.4	0.7	32.1±23.3	35.2±36.1	0.6
AUC <70 mg/dl	3.3±0.8	2.9±0.9	0.6	3.2±0.4	3.2±0.8	0.98

Hypo=hypoglycemia